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Live biotherapeutic products and their regulatory framework in Italy and Europe

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Abstract
In Italy and Europe, live microorganisms-containing products meant to be used by vulnerable or sick people for preventing or curing a disease are defined as live biotherapeutic products and are regulated as biological drugs. As such, they must undergo extensive quality, safety and efficacy testing and evaluation before receiving a marketing authorization. This review describes the regulatory framework of live biotherapeutic products with special focus on the European Pharmacopoeia monograph 3053 that set mandatory requirements for this kind of medicines, including verification of the number of live microorganisms and absence of certain contamination indicator microorganisms. The other product categories that may contain live microorganisms are also described, with brief references to the overlaps possibly occurring between the different categories.

Key words
- live biotherapeutic products
- regulatory requirements
- human microbiota
- safety

THE HUMAN MICROBIOTA AND THE POTENTIAL RELATIONSHIP WITH HEALTH STATUS

Over the past two decades, the application of omics technologies – whole genome sequencing, transcriptomics, metabolomics, and proteomics – to the study of human physiology and pathology, as well as to the understanding of microbial diversity, has gradually revealed the existence of a postnatally acquired organ within the human body, consisting of a resident complex microbial community [1, 2]. This microbial population includes eubacteria, archea, virus, fungi, yeasts and protozoa, in numbers that exceed at least 10 times those of the human body cells, and is commonly referred to as human microbiota, or microbiome when comprehensive of DNA agents such as phages and plasmids, proteins and metabolites, and the whole surrounding environment [3, 4]. It is partly established during birth by maternal vertical transmission; then, it further develops in early life through natural microbial colonization events that occur in the human body sites directly communicating with the outside, i.e., the gastro-intestinal tract, the skin, the naso-pharyngeal mucosa, the uro-genital mucosa, and the conjunctiva [5, 6]. From an ecological point of view, the human body and its microbiota evolve in a dynamic and mutualistic relationship, with the former providing an optimal niche for the bacteria to survive in, and the latter playing important roles in the maintenance of the host homeostasis [7, 8].

Defining the microbiota composition of the different human body sites in health and disease is currently an active research area. The focus of most studies is the intestine, as it is the largest interface with the external environment and the main reservoir of microorganisms in the human body, containing till 10^{14} bacteria of more than 1000 different species which approximately correspond to two third of the total human microbiota [9, 10].

Data indicate that the gut microbiota composition shows the highest intra- and inter-individual variability during the first three years of life, being shaped by various factors, such as delivery mode, diet (breast- or formula-feeding, introduction of solid food), genotype, environment, geographical and cultural factors, infections and use of antibiotics. Subsequently, it starts to resemble an adult-like composition that persists through childhood, adolescence and adulthood, although small changes constantly occur in relation to dietary modifications, hygiene, use of drugs, physical stress, etc. [4, 5, 11-13].

The sum of factors influencing the gut microbiota composition during the course of life accounts for the considerable variation of bacteria genera and species among individuals; however, the same bacteria phyla have been shown to prevail in all individuals, i.e., *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Protebacteria,*

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most strains being harmless commensals in the healthy human gut microbiota [14, 15].

A constant and balanced gut microbiota composition (eubiosis) provides positive effects to general health by directly contributing to metabolic functions, protection against pathogens and immune system stimulation [10, 11, 16-18]. Besides, there is increasing evidence of possible complex bidirectional signalling between the gut microbiota and other organs, such as the brain, the lung and the skin (generically defined as gut-brain, gut-lung and gut-skin axes), as indicated by the occurrence of intestinal complications during neurological, respiratory or skin disorders, and *vice versa* [10, 11, 19-21]. The bidirectional communication would be accomplished on one way by metabolites and endotoxins from the inflamed gut reaching the different organs via the blood circulation, and on the other way by products from the inflammatory processes in the organs acting on the gut microbiota [9, 11, 18].

In accordance, aberrant gut microbiota composition (dysbiosis) and the consequent imbalance of the host-microbes relationship may not only be directly implicated in the pathogenesis of gastrointestinal-related and autoimmune diseases, all characterized by over-responsiveness of the immune system, such as inflammatory bowel disease, colorectal cancer, obesity, type 2 diabetes, celiac disease, rheumatoid arthritis, etc. [5, 21]. In fact, dysbiosis is also increasingly recognized in *i)* brain and nervous system diseases (e.g., autism spectrum disorders, Alzheimer's disease), *ii)* lung diseases (e.g., asthma, cystic fibrosis, COVID-19 disease), and *iii)* skin disorders (e.g., psoriasis, atopic dermatitis), etc. [19, 20-23].

Microbial diversity loss with shift towards certain species has commonly been observed in the microbiota of diseased patients compared to those of healthy people, even though whether dysbiosis is the cause or rather the consequence of the disease often remains unclear [10, 24, 25].

Although less studied, the microbiota of body sites other than the intestine have also been hypothesized to play important roles in human health and disease: for instance, dysbiosis of the maternal vagina might be a risk factor for pre-term birth, while dysbiosis of skin, mouth or respiratory tract mucosa have been associated with conditions such as wound infection and acne, dental caries and other periodontal diseases, and sinusitis and pneumonia, respectively, just to name a few [20, 26-28].

CATEGORIES OF LIVE MICROORGANISMS-CONTAINING PRODUCTS FOR HUMAN USE

The increasing awareness of the important influence of eubiosis and dysbiosis in health and disease, has strongly promoted extensive research on the possibility of modulating the human microbiota for preventive and/or therapeutic purposes.

Consequently, the search for live microorganisms conferring health benefits to the human host has expanded exponentially, as well as their potential applications. In parallel, while the traditional delivery vehicles for live beneficial microorganisms were essentially fermented dairy products, they now include at least four different product categories, i.e., food and dietary supplements, medical foods, cosmetics and drugs *(Table 1)* [29-36]. Although live microorganisms may also be contained in biocides, this product category will not be discussed in this review as it is not intended for direct human use, but rather to control organisms that are harmful to human health [37]. The category of medical devices will not be considered as well, because live bacteria have expressly been excluded from medical devices according to recent European regulation [38].

All product categories of *Table 1* fall under distinct regulatory pathways in Europe.

This overview will specifically focus on the medicine category – referred to as "live biotherapeutic products" (LBPs), i.e., medicinal products containing live microorganisms as active substance(s) – and the related regulation in Italy and Europe, with brief reference to the other non-medicinal product categories regulations. Critical aspects as well as implementation issues will also be discussed.

LBPs AND THE NEED FOR THEIR REGULATION AND CONTROL

LBPs differ from the other product categories of *Table 1* because of the intended use, i.e., to prevent or cure a disease, and the intended target population, as they are aimed at individuals prone to develop a pathology or at sick people [39].

The American Food and Drug Administration (FDA) introduced the term "LBPs" in 2010 and subsequently specified, to clearly distinguish between the drug and food categories, that a product that is no longer used as a food with specific characteristics of nutritional content, taste and flavor, but for other physiological purposes, becomes a drug [40-42].

In 2019, the term LBPs was adopted by the European Pharmacopoeia (Ph. Eur.) that defined them as "medicinal products containing live microorganisms (bacteria or yeasts) for human use", and also detailed the possible administration routes (oral or vaginal) and the different types of pharmaceutical forms [34].

Similarly to the other product categories of *Table 1*, LBPs most frequently include – but are not limited to – lactic acid bacteria, bifidobacteria, bacilli and yeast strains, alone or in combination. As the same microbial or yeast strains can often be present in the different product categories, it has been suggested that, in order to stress the distinct categories purposes, different names should be applied to the beneficial microorganisms that they contain [43]. In this view, the term "probiotics", defined as "live microorganisms that when administered in adequate amounts confer a health benefit on the host" [32], would be restricted to the food and dietary supplements category, whereas alternative terms have been proposed for the microorganisms in LBPs, e.g., "biotherapeutic agents" or "pharmaceutical probiotics" or "pharmabiotics", although they have not yet been validated [35, 36].

The differentiation between the "probiotics" and "pharmabiotics" terms has relevant implications for safety assessment. In fact, the main pre-requisite to

Table 1
Product categories that may contain live beneficial microorganisms

PRODUCT CATEGORY	Product characteristics and definitions (according to EU legislation)	Live beneficial microorganisms designation	Mode of administration	Target population	Intended use
Food	Food and beverages Dietary supplements Infant formula	Probiotics[a]	Oral	Healthy people	To retain and improve health and well-being
Medical food (Foods for special medical purposes)	Food specially processed or formulated and intended for the dietary management of patients, including infants, to be used under medical supervision [b]	Probiotics[a]	Oral or enteral, under the supervision of a physician	Patients with a disease that requires dietary management	Specific dietary management of a disease that has distinctive nutritional needs
Cosmetic products	Any substance or mixture intended to be placed in contact to the external parts of the human body (epidermis, hair, nails, lips, teeth) [c]	Probiotics[a]	Topical	Healthy people	Care of skin, hair, teeth, nails Cleaning Keeping in good condition
Drugs (Live biotherapeutic products, LBPs)	Medicinal products containing live microorganisms (bacteria or yeasts) for human use [d]	Pharmabiotics[e]	Oral or vaginal	Sick people or people prone to develop a pathology	To treat or prevent a disease

[a] [32], [b] [33], [c] [30], [d] [38], [e] Proposed (not yet validated) definition [35, 36].

qualify live microorganisms in foods as probiotics – provided their correct and unequivocal identification and accurate characterization – is their long history of safe use, implying consumption by healthy individuals; conversely, each pharmabiotic microorganism in pharmaceutical products requires a proper safety assessment, including *in vitro* and *in vivo* studies, and clinical trials in human volunteers [31, 44-46].

Despite the recommendation of maintaining the different terms, the term "probiotics" is still often used to include the microorganisms contained in LBPs as well as in the other product categories of *Table 1*, thus causing misleading overlaps between products, as discussed later in the text.

The intended use and target population are indeed the main focal points for the regulatory status of a certain product category [39, 40].

At the European level, the Directive 2004/27/EC – amending the Directive 2001/83/EC – describes a *medicinal product* as "(a) any substance or combination of substances having properties for treating or preventing disease in human beings and (b) any substance or combination of substances which can be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action or to making a medical diagnosis" [47].

Falling within this definition, LBPs are to be considered medicinal products and, as such, they can specifically rely on regulatory concepts available for other biological medicines – including vaccines, blood and plasma-derived medicinal products, and advanced therapy medicinal products – requiring to undergo rigorous evaluation of quality, safety and efficacy, in order to obtain a marketing authorization (MA).

Quality aspects such as batch-to-batch consistency, the influence of upscaling process on yield and potency, and the stability of the final product should be closely monitored during production of LBPs. As for other pharmaceutical products, the quality of LBPs must be ensured during pharmaceutical development and production through the application of good manufacturing practices (GMPs); their clinical efficacy should be confirmed by independent trials of acceptable quality (double-blinded, randomized, placebo-controlled trials), performed with specific lots produced under GMP conditions, and applied to a well-defined patient population, using established treatment conditions, dosage, and defined and validated primary endpoint(s).

Challenges to the evaluation process of LBPs may be related to issues such as animal-to-human translation and/or to the suitability of certain preclinical animal models.

As with any drug, also for LBPs the safety assessment is based on a risk analysis which primarily involves the identification of risks and the assessment of the probability of their occurrence and impact. On this basis, the risk must be managed by adopting measures to avoid, mitigate or accept its effects, and constantly monitored over time.

However, as above mentioned, the safety evaluation of LBPs needs further special consideration compared to traditional medicines, due to the peculiar biological properties and mode of action of the live microorganisms that they contain [48].

THE SAFETY CONCERNS RELATED TO LIVE MICROORGANISMS FOR HUMAN USE

Two main safety concerns have been raised on the intentional use of large amounts of live microorganisms:

a) the possibility of adverse side effects due to their translocation into the blood circulation, particularly in higher risk people, and *b)* their potential role as reservoir of antibiotic resistance genes or putative virulence/toxin genes [49-51]. While there is growing evidence on the occurrence of severe infectious adverse events in vulnerable populations, the second safety aspect is more theoretical since so far it has not been supported by clinical evidence but only by *in vitro* and *in vivo* studies.

a. Infectious complications associated to the use of live microorganisms

A large number of randomized double-blind placebo controlled human trials have been carried out to prove the efficacy of probiotics, as recommended by the FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Foods [52, 53]. However, most studies have been conducted with healthy individuals, while only a few assessed their safety for vulnerable groups of people, such as critically ill or immunocompromised patients, elderly people, premature infants, pregnant women, etc.; moreover, it has been noted that even when the target population was vulnerable people, adverse events were not adequately assessed in the follow-ups of clinical trials and/or insufficiently reported or documented [45, 53-56]. For these reasons, the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) has recently recommended that any adverse events specifically linked to the consumption of live microorganisms-containing products should be appropriately recorded in a dedicated register maintained by health authorities [57].

Notably, for a proper drug safety assessment, at least two clinical trials with the same primary endpoint should be independently conducted in distinct centers [58].

As a matter of fact, the administration of large amounts of live microorganisms to vulnerable people, often in combination with or after excessive use of antibiotics, has occasionally been correlated to infectious complications in the treated patients: *Table 2* describes the microorganisms most frequently associated with infectious complications and the underlying conditions identified in the vulnerable population groups, according to most recently published systematic reviews, meta-analyses and single case reports [45, 50, 54, 56, 59-74].

In several cases, the microorganism isolated from the patient was shown by molecular techniques to be identical to the one administered, confirming that it was indeed the infection source [66, 67, 70-74].

Leaking intestinal mucosa barrier and/or immunosuppression in the vulnerable individuals have been suggested as possible predisposing factors contributing to the infectious progression, even though the underlying mechanisms remain unclear [24].

The adhesion properties of the microorganisms, that are normally a selection criteria for probiotics, allowing them to increase persistence and colonization in the host gut, but also critical for microbial pathogens, have been hypothesized to act as a virulence factor in vulnerable individuals, by increasing the microorganism translocation from the intestine to the blood stream [50, 58].

Of note, several cases of fungemia occurring in intensive care units, mainly due to the yeast *Saccharomyces cerevisiae var. boulardii,* were caused by the accidental contamination of the central intravenous catheter of hospitalized patients who were either being treated with preparations containing that microorganism or were infected by spread from other roommate(s) who were receiving such preparations. *S. cerevisiae var. boulardii* is indeed a spore-forming yeast rapidly spreading into the environment, especially in the absence of high standard hygienic practices [70, 73-75].

Although in most studies no deaths were reported, others describe variable mortality rates, even though whether death had resulted from the infectious complications due to the treatment or from the underlying conditions of the patients was unclear in some cases: in general, the factors associated with higher mortalities were the severity of patient conditions and immunosuppression or the presence of co-morbidities, while lower mortalities were associated to prompt therapy with effective antibiotics [50, 54, 56, 59, 60].

It must be highlighted that the incidence of infectious complications related to the use of live microorganisms in vulnerable people appears to be extremely low and

Table 2
Infection cases associated with so-called "probiotic therapy", often coupled with antibiotic therapy[a]

Microorganisms involved (in order of decreasing frequency)	Underlying conditions of treated patients (in order of decreasing frequency)
• *Saccharomyces spp* [b] *(S. cerevisiae var. boulardii)* • *Lactobacillus spp. (L. rhamnosus, L. delbruecki subsp. bulgaricus, L. acidophilus, L. paracasei, L. reuteri, L. gasseri)* • *Bifidobacterium spp. (B. breve, B. longum subsp. infantis)* • *Bacillus spp. (B. clausii)* • *Lactococcus spp. (L. lactis)*	• Immunocompromised patients often admitted to ICU (cancer, organ transplantation, surgical intervention, AIDS, hepatic cyrrhosis, diabetes, etc.) • Pre-term newborns or newborns with pathological conditions • Healthy seniors • Children with short gut syndrome • Non-immunocompromised people suffering from diarrhea • Non-immunocompromised people suffering from ulcerative colitis • Non-immunocompromised people suffering from dental abscess

[a][45, 50, 54, 56, 59-74], [b]Infections either caused by direct ingestion or catheter contamination.

largely exceeded by the reported positive effects of the same live cultures in healthy population [50, 54, 76].

In any case, although the studies are very heterogeneous for characteristics of the treated population groups (age, illness, received therapy previous to treatment, etc.), live microorganisms that were used, and dose and duration of treatments, altogether the results clearly indicate an increased risk of developing infections in the vulnerable groups treated with the live microbial preparations compared to the untreated patients.

Thus, despite overwhelming evidence that live probiotic microorganisms are effective and can safely be taken by healthy individuals, a careful analysis of the risk-benefit ratio should be applied by clinicians before recommending any products containing live microorganisms, either a food, a supplement or a medicine, to vulnerable patients, who on their side should be fully informed prior to treatment. In particular, measures to ensure the safe handling and administration of live microorganisms-based formulations to hospitalized and seriously ill patients should be implemented by the hospital staff.

b. Potential transfer of antibiotic resistance genes or putative virulence/toxin genes

Antibiotic resistance (AR) could be a selective advantage for probiotic/pharmabiotic microorganisms, especially those used in combination with antibiotics to re-equilibrate the intestinal microbiota, as they would have better chances to survive, colonize the host gut and also contemporarily exclude other antibiotic-resistant bacteria by competition; on the other hand, given that the gut microbiota is one of the main hot spots for horizontal gene transfers, the concomitant presence in the intestine of broad-spectrum antibiotics and antibiotic-resistant live microorganisms might exert a selective pressure potentially leading to AR spread to opportunistic pathogenic bacteria strains, thus seriously reducing the therapeutic treatments of infections [51, 77, 78].

For this reason, demonstration that the AR is intrinsic (non-transferable), and not extrinsic (acquired) is required for both probiotics and pharmabiotics by the respective EU regulating authorities, i.e., EFSA and Ph. Eur. [34, 79]. In particular, the AR genes should not be located within potentially mobile genetic elements such as plasmids, transposons, integrons, and bacteriophages in order to exclude the possibility that they are horizontally transferred from probiotics/pharmabiotics to commensal or pathogenic bacteria, or *viceversa*.

Several studies, some of which using metagenomics approaches, have shown that probiotic strains may harbor AR genes [51, 80-83]: yet, the presence of AR genes in a microorganism does not always result in resistance, since point mutations, deletions or insertions in the genes could negatively affect the gene expression. Thus, concurrent testing for phenotypic resistance would be appropriate: however, another challenge is posed by the fact that the minimum inhibitory concentrations cut-off values for most antibiotics used to test the probiotic strains are not always available or standardized [51, 78].

Nonetheless, neither detection of AR genes nor phenotypic evaluation of AR indicate whether the AR genes may indeed be transferable.

The location of AR genes within potentially mobile genetic elements, such as plasmids or transposons, which could contribute to spread, has been demonstrated in strains belonging to typical probiotic/pharmabiotic genera, including lactobacilli and bifidobacteria strains [80, 83, 84].

More importantly, a number of studies have shown the transfer of AR genes between microbial genera commonly used for human (or animal) use (especially lactic acid bacteria) and pathogenic strains. For instance: (a) the tetracycline *tet*(M) resistance gene could be horizontally transferred *in vitro* from some *Lactobacillus spp*. (namely, *L. plantarum*, *L. sakei* and *L. alimentarius*) to *Enterococcus faecalis* and *Lactococcus lactis*, and from *L. delbruecki bulgaricus* to *Listeria monocytogenes* [85]; (b) the erythromycin (*ermB*) resistance gene was shown to transfer from *L. plantarum* to *E. faecalis* in the gastrointestinal tract of rats [86]; (c) the genetic transfer of the vancomycin (*vanA*) resistance and ampicillin (*amp*) resistance genes between probiotic strains of *Enterococcus faecium* and probiotic strains of *L. acidophilus* has been demonstrated *in vivo*, in the gut of mice [87].

The potential dissemination of toxin/virulence genes among probiotic/pharmabiotic microorganisms is also of concern, as it could lead to the emergence of bacteria or yeast strains with new pathogenic potentials. In fact, similarly to the AR genes, the genes encoding any putative pathogenic factors (such as adhesion factors, cytolysins, damaging enzymes, and biogenic amines) could be transferred through mobile genetic elements between commensal and pathogenic bacteria and probiotic/pharmabiotic microorganisms, in response to any environmental pressure within the dynamic microbiota of the different human body sites [51, 77, 88].

Higher awareness of the above-described critical safety aspects potentially related to the consumption of probiotics/pharmabiotics is necessary to lead to more careful advising by clinical professionals and more prudential purchasing by consumers; it also demands for increasing regulation and control of the products that contain live microorganisms.

Ph. Eur. MONOGRAPH 3053

In Europe, testing and compliance to the standards detailed within the Ph. Eur. compendia is a basic requirement for the manufacturing and release of pharmaceutical ingredients and drug products.

In 2019, Ph. Eur. released a general monograph on *Live biotherapeutic products for human use* (3053), as well as two accompanying general chapters: *Microbial examination of live biotherapeutic products (LBP): test for enumeration of microbial contaminants* (2.6.36) and *Microbiological examination of live biotherapeutic products: test for specified microorganisms* (2.6.38) [34, 89, 90].

In particular, the Ph. Eur. monograph 3053 describes the quality and safety requirements for LBPs during production and in the finished lots [34].

These requirements specifically concern (a) the pro-

duction method and (b) the microorganisms (bacteria or yeasts) used:

(a) the production method must ensure constant final yields and microbial viability maintenance through the whole process; furthermore, the number of microbial subcultures from the master seed lot must not exceed that used for LBP production and shown to be satisfactory in the clinical trials. Consistent minimization or removal of any impurities or adventitious agents is also required during production;

(b) the microorganism(s) to be used must accurately be identified, and characterization at the strain level is required: the origin of strain(s), any subsequent manipulations, and description of the culture media used to grow the microorganism(s) should be provided. The tests used to characterize the phenotype and genotype of strain(s) must be detailed, and the stability of phenotypes and genotypes demonstrated. As above mentioned, besides the determination of their antibiotic susceptibility, the absence of any antibiotic resistance genes potentially transferable to the human microbiota is required. Furthermore, any virulence factor in the microorganism "should be investigated and evaluated with respect to safety".

As an additional quality requirement for those products to be orally administered, survivability of the microbial strain(s) in the human gut must be demonstrated by *in vitro* gastric acid and bile resistance testing.

Since microbial seed lot system is required to be used during production, it is recommended that it contains no adventitious agents or other impurities, and that any replacing seed lots are fully characterized. The sterility of the culture media must be ensured along with the absence of ingredients known to cause toxic, allergic or other undesirable effects; if inclusion of such ingredients is necessary, demonstration that the residual amount in the final lot does not affect product safety must be provided. Growth and harvesting must be performed under appropriate conditions. Stability data should be established for each intermediate product.

Tests to be performed on the final lots include the identification of each microorganism by proper methods, as well as the determination of the number of each live microorganisms (potency) by a suitable microbial enumeration test, and expressed as CFU/g, CFU/ml, CFU/unit: the resulting number must not be less than the stated range.

Concerning the microbiological quality of LBPs, the monograph 3053 indicates the acceptance criteria for aerobic microbial contamination counts (AMCC) and yeast and moulds contamination counts (YMCC) for all LBP products, whereas absence of *Escherichia coli* in those to be orally administered, and of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* in those for vaginal use is required.

The appearance, and the pH and water activity values of the final lots must comply with the specifications established for the products.

Regarding the storage conditions, it is recommended that liquid LBPs are not frozen, in order to maintain the full viability of the microorganisms.

Labels must state the name of strains, the potency of each strain (expressed as CFU/g, CFU/ml/CFU/unit or as viable cells/ml), the route of administration, the storage conditions, the expiry date, the name of any stabilizers and other excipients. For freeze-dried preparations, labels must also state the indications on reconstitution before use with name, composition and volume of the liquid to be added, and finally the storage conditions and expiration after the product has been reconstituted [34].

THE REGULATORY SITUATION FOR LBPs IN ITALY

As for other medicinal products, in order to obtain a MA for LBPs in Italy and Europe, the company has to submit an application, consisting of a dossier containing information on the chemical-pharmaceutical, preclinical and clinical aspects, structured according to a standardized format (CTD – Common Technical Document). The data and studies submitted to support the application for MA must comply with guidelines defined at international level (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use – ICH M4 document) [91]. The CTD includes 5 modules, of which module 1 contains the administrative information and prescribing information; module 2, the information summaries; module 3, the quality characterization of the product; module 4, the non-clinical study reports; and module 5, the clinical study reports.

Importantly, the CTD must provide the information concerning the product characteristics, labelling and package leaflet. Moreover, application of the good manufacturing practices (GMPs) according to the Commission Directive EU 2017/1572 during the product manufacturing must be demonstrated in the CTD [92].

According to the European legislation, LBPs can apply to a MA by either a National authorization procedure or by European procedures, the latter involving several EU regulatory authorities in the scientific assessment of the CTD provided by the applicant. Currently, all LBPs marketed in Italy have been licensed at national level.

For most of them, the MA was issued before the implementation of Directive 2001/83/EC, but during the renewal procedure submitted after the national transposition of this directive in 2006, the LBPs underwent a critical review, in order to achieve compliance of the authorization in Italy with the requirements of the European Union legislation.

LBPs AND PROBIOTIC SUPPLEMENTS: SIMILARITIES AND OVERLAPS

Despite the sharp demarcation existing between the regulations of the food/dietary supplements, medical foods, cosmetics and drugs categories *(Table 1)*, overlapping of products still occurs, most frequently between LBPs (drugs) and probiotic food supplements.

At the EU level, the food supplements fall within the scope of the general food law, i.e. Regulation (EU) 2017/625 [93]. With specific regard to the microorganisms contained in the supplements, EFSA has released

Table 3
Comparison of products containing the same microorganism (*Saccharomyces boulardii*) in similar amounts that are marketed in Italy either as LBPs or as probiotic supplements

Product features	LBP	Probiotic supplement
Information to patients	Patient information leaflet: - included in the medicine package; - publicly available, together with the Summary of Product Characteristics, on the web site of the Italian Medicines Agency (AIFA)[a]	No patient information leaflet included in the supplement package The product is listed in the Italian Register of dietary supplements[b], but information is only available on the manufacturer website
Product form	Capsule	Capsule
Product content	Each capsule contains: - Active substance: *S. boulardii,* 5 billion live microorganisms - Excipients: lactose, magnesium stearate, gelatin, titanium dioxide	Two capsules contain: - *S. boulardii* (MYA796), 10 billion; - *Enterococcus faecium* (SGEf01), 2 billion; - Magnesium hydroxide 124 mg; Zinc 4 mg.
Indications	- Prophylaxis and treatment of intestinal dysmicrobism and disvitaminosis due to antibiotic use - Prophylaxis and treatment of traveler's diarrhea - Treatment of acute diarrhea of different origins - Treatment of Irritable Bowel Syndrome - Treatment of candidiasis of the gastrointestinal tract	- Restoration of the intestinal flora equilibrium, specially following antibiotic or chemotherapeutic treatments - Zinc contributes to normal activity of the immune system
Dosage and use	- 1 or 2 capsules, twice daily - Do not take with hot or alcoholic drinks - capsule must be swollen intact	- 2 capsules daily, before or during meals - For children under six years, the content of the capsule can be solubilized in water or in other drinks
Warnings	Do not take the product if you: - are allergic to *S. boulardii* or other ingredients contained in the medicine - are allergic to yeast - are under treatment with anti-fungal/mycosis medicine - are an immunocompromised patient or if you are in hospital	- Do not exceed the recommended dose of capsules - The food supplements are not intended to replace a balanced diet - Keep out of reach of children under three years

LBPs: live biotherapeutic products.
[a]https://farmaci.agenziafarmaco.gov.it/bancadatifarmaci/home.
[b]https://www.salute.gov.it/portale/temi/p2_6.jsp?id=3668&area=Alimenti+particolari+e+integratori&menu=registri.

Table 4
Example of products with similar brand names from the same company that are sold in Italy as live biotherapeutic products (LBPs) or as probiotic supplements (PSs)

Product brand name (LBP or PS)	Microorganisms and quantities Other active principles	Indications
X[a] (LBP)	*Bacillus clausii* poly-antibiotic-resistant spores (strains SIN, O/C, T, N/R) 1 billion	- Prevention and treatment of intestinal disorders related to alterations of the intestinal microflora causing diarrhoea, abdominal pain and disvitaminosis - Restoration of the intestinal microflora during antibiotic or chemotherapy treatments - Treatment of acute or chronic gastrointestinal diseases of breast-fed children
X-A[b] (PS)	*Bacillus clausii* (strain SIN) 6 billion Zinc, Selenium	Restoration of the intestinal microflora balance
X-B[b] (PS)	*Lactobacillus acidophilus* strain LA-5 1 billion *Bifidobacterium animalis subsp. lactis* strain BB-12 2 billion Mint and coriander extracts	Fight abdominal swelling and tension, aerophagia, and imbalance of the intestinal microflora
X-C[b] (PS)	*Bifidobacterium lactis* strain HN019 ATCC SD5674 1 billion Fructo-oligosaccharides (FOS)	Restoration of the intestinal microflora balance and facilitation of the intestinal transit
X-D[b] (PS)	*Saccharomyces boulardi* 6 billion Vitamins A, D, B6, B9 and B12	Restoration of the intestinal microflora balance

[a]X: same name for the five (LBP and PS) products.
[b]A, B, C, D: specific designations differentiating the PS products on the bases of the claimed functions.

and regularly updates a list of microorganisms that are given the "quality presumption of safety" (QPS) status based on long tradition of safe use, absence of virulence/pathogenic factors which could harm the host, and absence of any potentially transferable antibiotic resistance genes [94, 95]. The accurate identification and characterization of the microorganisms is necessary to fulfill all requirements. The QPS approach was developed to provide a safety pre-assessment of microorganisms to be intentionally added to food and feed: since the QPS status is granted at the species level for bacteria and yeasts, it is recommended that any safety concern be excluded at the strain level [95]. As an example, the *Enterococcus faecium* species is excluded from the QPS evaluation because some of its members have been associated to nosocomial infections: however, certain beneficial *E. faecium* strains that are sensitive to the ampicillin antibiotic and do not contain any genetic elements commonly found in the nosocomial strains, as required by EFSA, may be used in food and feed supplements, as well as in medicines [95-97].

Furthermore, Italy and some other EU countries have set national guidelines on probiotics and prebiotics complementing the European regulations: for example, the Italian guidelines propose that the identification and characterization of bacteria and yeasts is always made at the strain level [98]. In addition, the Regulation (EC) No 1924/2006 on nutrition and health claims made on food also applies to the supplements containing live microorganisms [99]. According to the interpretation of this regulation by the EU Commission, the term "probiotic" is a health claim, as it implies a beneficial effect on health. This could be misleading for consumers if "probiotic" is used on labels, unless sustained by adequate scientific evidence. As a result of this interpretation, since 2007 EFSA has only approved the use of the term "probiotic" for a single yogurt containing *Streptococcus thermophilus* and *Lactobacillus delbruecki subsp. bulgaricus* (minimum 10^8 CFU/g) for which the claim "it improves lactose digestion" was satisfactorily demonstrated [100]. Since this interpretation of Regulation (EC) No 1924/2006 was increasingly deemed too restrictive, in recent years divergent interpretations on the use of the "probiotic" term were given in the EU Member States: consequently, in some countries including Italy its use has been allowed in the labeling and advertising of food and supplements, provided that specific conditions are fulfilled [98].

In summary, similarly to LBPs, the probiotic supplements are also regulated by complex rules, the former as drugs and the latter as foods.

Several aspects of the two distinct food and drug regulations are comparable, such as most requirements for live microorganisms and their quantities in the products. However, a remarkable difference is that LBPs must undergo extensive pharmacodynamics, pharmacokinetic, safety and efficacy testing and evaluation prior to receiving MA, while in most countries including Italy the probiotic supplements, as other dietary supplements, are not subjected to any pre-marketing authorization. In fact, they just need to be notified to the competent health authority that evaluates the general compliance of the product content and label with the food legislation (the Italian Register of dietary supplements is available at: www.salute.gov.it/portale/temi/p2_6.jsp?id=3668&area=Alimenti+particolari+e+integratori&menu=registri).

The fact that the procedure to get MA is much faster for probiotic food supplements compared to LBPs clarifies the reason why the probiotic food supplements largely exceed LBPs in the market, and is also at the basis of some drawbacks.

For instance, in Italy products of different brands containing the same microorganism(s) in similar quantities may actually be sold over the counter in the drugstores either as LBPs or as probiotic supplements, with the latter being easily available in the grocery shops, too *(Table 3)*. This implies that less information on the product characteristics and on safety warnings are available for the probiotic supplements compared to the LBPs counterparts, because the specific requirements for labeling and package leaflets that apply to LBPs are not needed for supplements, and this could represent a risk for diseased costumers purchasing them.

In Italy, this scenario is further complicated by the fact that some LBPs and probiotic supplements from the same company are marketed with very similar brand names, despite the fact that they contain distinct microorganisms and/or different quantities of the same microorganism, and are intended for different purposes *(Table 4)*.

CONCLUSIONS

In this review, considerable differences have emerged between the regulatory frameworks of LBPs and probiotic food supplements, even though both products and the related regulations also share several similarities, including frequently the same microorganisms in similar amounts. It must be mentioned that in Italy both LBPs and probiotic food supplements are sold over the counter in the drug stores, with difficulties in distinguishing between them for most customers and sometimes for the physicians, too. For these reasons, further improvement and harmonization between the regulations of LBPs and probiotic food supplements would merit consideration. This would properly orient the physicians and increase end users confidence besides implementing research in the field and ultimately supporting the manufacturers to invest in new products development.

Authors' contributions

All Authors conceived the study. GF wrote the manuscript, CvH critically revised the draft, MJG and SG contributed to the implementation of the final version of the manuscript.

Conflict of interest statement

The Authors declare that they have no conflict of interest.

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